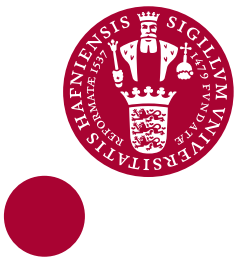

Effect of prenatal bisphenol A exposure on early childhood body mass index through epigenetic influence on the insulin-like growth factor 2 receptor (IGF2R) gene

Choi, Yoon-Jung; Lee, Young Ah; Hong, Yun-Chul; Cho, Jinwoo; Lee, Kyung-Shin; Shin, Choong Ho; Kim, Bung-Nyun; Kim, Johanna Inhyang; Park, Soo Jin; Bisgaard, Hans; Bønnelykke, Klaus; Lim, Youn-Hee

Published in:
Environment International

DOI:
10.1016/j.envint.2020.105929

Publication date:
2020

Document version
Publisher's PDF, also known as Version of record

Environment International 143 (2020) 105929

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Effect of prenatal bisphenol A exposure on early childhood body mass index through epigenetic influence on the insulin-like growth factor 2 receptor (*IGF2R*) gene

Yoon-Jung Choi[a,b,1], Young Ah Lee[c,1], Yun-Chul Hong[a,b,d], Jinwoo Cho[d], Kyung-Shin Lee[a,b], Choong Ho Shin[c], Bung-Nyun Kim[e], Johanna Inhyang Kim[f], Soo Jin Park[g], Hans Bisgaard[h], Klaus Bønnelykke[h], Youn-Hee Lim[d,i,*]

[a] Department of Preventive Medicine, Seoul National University College of Medicine, Seoul 03080, Republic of Korea
[b] Environmental Health Center, Seoul National University College of Medicine, Seoul 03080, Republic of Korea
[c] Department of Pediatrics, Seoul National University College of Medicine, Seoul 03080, Republic of Korea
[d] Institute of Environmental Medicine, Seoul National University Medical Research Center, Seoul 03080, Republic of Korea
[e] Division of Children and Adolescent Psychiatry, Department of Psychiatry, Seoul National University Hospital, Seoul 03080, Republic of Korea
[f] Department of Psychiatry, Hanyang University Medical Center, Seoul 04763, Republic of Korea
[g] Department of Surgery, Wonkwang University Sanbon Hospital, Gunpo 15865, Republic of Korea
[h] COPSAC (Copenhagen Prospective Studies on Asthma in Childhood), Herlev and Gentofte Hospital, University of Copenhagen, 2820, Gentofte, Copenhagen, Denmark
[i] Section of Environmental Epidemiology, Department of Public Health, University of Copenhagen, Copenhagen 1014, Denmark

ARTICLE INFO

Handling Editor: Adrian Covaci

Keywords:
Bisphenol A
Prenatal
DNA methylation
Epigenetics
Child
Body mass index

ABSTRACT

Objectives: Epigenetic mechanisms have been suggested to play a role in the link between *in utero* exposure to bisphenol A (BPA) and pediatric obesity; however, there is little evidence regarding this mechanism in humans. We obtained data on obesity-associated CpG sites from a previous epigenome-wide association study, and then examined whether methylation at those CpG sites was influenced by prenatal BPA exposure. We then evaluated the relationship between CpG methylation status and body mass index (BMI) in a prospective children's cohort at ages 2, 4, 6, and 8 years.

Methods: Methylation profiles of 59 children were longitudinally analyzed at ages 2 and 6 years using the Infinium Human Methylation BeadChip. A total of 594 CpG sites known to be BMI or obesity-associated sites were tested for an association with prenatal BPA levels, categorized into low and high exposure groups based on the 80th percentile of maternal BPA levels (2.68 μg/g creatinine), followed by an analysis of the association between DNA methylation and BMI from ages 2–8.

Results: There was a significant increase in the methylation levels of cg19196862 (*IGF2R*) in the high BPA group at age 2 years ($p = 0.00030$, false discovery rate corrected $p < 0.10$) but not at age 6. With one standard deviation increase of methylation at cg19196862 (*IGF2R*) at age 2 years, the linear mixed model analysis revealed that BMI during ages 2–8 years significantly increased by 0.49 (95% confidence interval; 0.08, 0.90) in girls, but not in boys. The indirect effect of prenatal BPA exposure on early childhood BMI through methylation at cg19196862 (*IGF2R*) at age 2 years was marginally significant.

Conclusions: Prenatal exposure to BPA may influence differential methylation of *IGF2R* at age 2. This result indicates that a possible sensitive period of DNA methylation occurs earlier during development, which may affect BMI until later childhood in a sex-specific manner.

Abbreviations: ACME, average causal mediation effect; ADE, average direct effect; BMI, body mass index; BMIQ, beta mixture quantile; DOHaD, developmental origin of health and disease; EDC, environment and development of children; EWAS, epigenome-wide association studies; FDR, false discovery rate; FFQ, food frequency questionnaires; ICC, intraclass correlation coefficient; LOD, limit of detection; NA, not-available; NK, natural killer; SD, standard deviation; SNP, single nucleotide polymorphism

* Corresponding author at: Section of Environmental Epidemiology, Department of Public Health, University of Copenhagen, Øster Farimagsgade 5, 1014 København K, Denmark.
E-mail address: younhee.lim@sund.ku.dk (Y.-H. Lim).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.envint.2020.105929
Received 29 January 2020; Received in revised form 22 June 2020; Accepted 23 June 2020

1. Introduction

Bisphenol A (BPA) is one of the most synthesized chemicals worldwide, and is used in industry to manufacture polycarbonate plastics and resins, thermal merchandise receipts, food cans, dental fillings, and medical equipment (Braun 2017). BPA is ubiquitously found in polycarbonate water bottles, food storage containers, and water supply pipes. Humans are mainly exposed to BPA through oral ingestion, but can also be exposed via inhalation of dust or dermal contact with thermal receipts (von Goetz et al. 2010). BPA has been widely detected not only in human urine samples in various populations, including pregnant women and children (Myridakis et al. 2015; Romano et al. 2015; Vandenberg et al. 2010), but also in the placenta (Balakrishnan et al. 2010), amniotic fluid, and human breast milk (Vandenberg et al. 2007), suggesting *in utero* BPA exposure and continuous postnatal exposure in childhood.

Prenatal exposure to BPA has been indicated as an obesogen in pediatric epidemiological studies, although the results are inconsistent, depending on the sex and age at adiposity measurement. In the case of boys, Vafeiadi et al. (2016) found a positive association between prenatal BPA exposure and body mass index (BMI)-Z score only in boys at age 4, while Hoepner et al. (2016) and Harley et al. (2013) reported null effects of BPA on BMI Z-score in boys at age 7. For girls, Hoepner et al. (2016) found a positive association between prenatal BPA exposure and fat mass index and waist circumference (WC) in girls only at age 7, while Vafeiadi et al. (2016) and Harley et al. (2013) showed a negative association between BMI Z-score and percent body fat at age 9.

Animal studies have shown the effect of maternal BPA exposure on DNA methylation and obesity in a sex-specific manner. *In utero* and lactational exposure to BPA in mice results in sex-dependently altered metabolic phenotypes in offspring, in which male mice showed a dose-dependent increase in body and liver weights, while female mice showed a dose-dependent decrease in body, liver, fat pad weights, and adipocyte sizes (van Esterik et al. 2014). Since the association between prenatal BPA and obesity-related phenotypic changes is more consistent in female mice, the methylation patterns were further analyzed in female mice, which showed differential methylation patterns in the liver tissue of female offspring (van Esterik et al. 2015).

In a cohort study of children, the second and highest tertile of maternal urinary BPA during the second trimester is associated with higher *IGF2* methylation in children at age 14 compared to the lowest tertile (Goodrich et al. 2016), although it was not investigated whether such methylation changes led to body weight changes. Another epigenetic epidemiological study showed that *in utero* exposure to BPA is related to hypomethylation in the *MEST* gene promoter in cord blood as well as increased expression of *MEST*, which in turn, is associated with an increase in the BMI Z-score in children (Junge et al. 2018), although sex-dependent effects were not shown. Obesity-related methylation changes have been reported in a few Eepigenome-wide association studies (EWAS) in children (Huang et al. 2015; Rzehak et al. 2017; Sharp et al. 2017); however, it remains unknown whether such methylation changes are related to early life environmental exposures.

We aimed to identify whether differentially methylated CpG sites resulted from prenatal BPA exposure and whether such methylation differences led to sex-specific obesity in early childhood. We used data from a prospective children's cohort study including prenatal BPA exposure, methylation assessment at ages 2 and 6, and longitudinal BMI data from ages 2, 4, 6, and 8. Since we were interested in prenatal BPA exposure and early life methylation changes, specifically associated with obesity, we focused on analyzing targeted CpG sites from previous obesity-related EWAS rather than exploring the whole epigenome.

2. Methods

2.1. Study participants

We used data from the Environment and Development of Children (EDC) Study, an ongoing prospective cohort study designed to evaluate the effects of early-life environmental exposures on physical and neurobehavioral development, as described previously (Kim et al. 2018). A total of 726 children [2-year old (n = 425) and 4-year-old (n = 301) children] were firstly recruited for the EDC cohort during 2012–2015, who were born from mothers participating in the Congenital Anomaly Study cohort, and followed-up at 2-year intervals. Mothers' urine samples were collected during the second trimester of pregnancy, although cord blood was not collected. In this sub-study, methylation profiles were analyzed at ages 2 and 6 years in 59 children among those who visited from ages 2, 4, 6, to 8 years. Informed consent was obtained in line with the Institutional Review Board of Seoul National University College of Medicine (IRB No. 1201-010-392).

2.2. Outcome definitions

Height was measured using a Harpenden stadiometer (Holtain Ltd., Crymych, Wales, UK) and weight was measured using a digital scale (150 A; Cas Co. Ltd., Seoul, Korea) by trained personnel. BMI was calculated as weight/height^2 (kg/m^2). The Z-scores for height, weight, and BMI were assigned on the basis of the 2007 Korean National Growth Charts (Moon et al. 2008). An extensive questionnaire including birth history, family history, past medical history, socioeconomic status, lifestyle factors including diet, and environmental toxicant exposure in mothers and children, was completed by the parents at every visit.

2.3. Prenatal exposure

Urine BPA was measured as described in a previous study (Lim et al. 2017a). The first void urine in the morning was collected in conical tubes (SPL Lifesciences, Pocheon, Gyunggii-do, Korea) from mothers and children after 8 hours of fasting. Maternal urine was collected during the second trimester of pregnancy (15–27 weeks, mean 20 weeks of gestation), and the samples were stored at −20 °C (Seegene Medical Foundation, Seoul, Korea). Total BPA concentrations, including free and conjugated species, were measured, and the conjugated BPA species were hydrolyzed with β-glucuronidase/sulfatase. BPA concentrations were quantified by high-performance liquid chromatography-tandem mass spectrometry (Agilent 6410 Triple Quad LCMS; Agilent, Santa Clara, CA, USA) (Yang et al. 2003). Standard BPA solutions were prepared at 50, 25, 12.5, 6.25, 3.125, and 1.5625 μg/L, and analyzed using a blank to determine the standard calibration curve ($r^2 > 0.999$). When the measured concentration of a sample was above the maximum level of the standard BPA solution, the sample was diluted (1:1) and re-analyzed. The sample was also re-analyzed if the detected concentration was not within 20% of the standard calibration curve. The lower limit of detection (LOD) ranged from 0.031 to 0.212 μg/L, depending on the batch, and we used a LOD value of $0.212/\sqrt{2}$. We used creatinine-adjusted BPA values in units of μg/g creatinine throughout our analysis (Calafat et al. 2005).

2.4. DNA methylation

2.4.1. Assessment of DNA methylation

DNA methylation was measured in whole blood samples repeatedly obtained at ages 2 and 6 from 59 children. The quality of the DNA samples was checked using the NanoDrop® ND-1000 UV–Vis Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). After electrophoresis on a 1% agarose gel, samples with intact genomic DNA (gDNA) were selected, and then diluted to 50 ng/μL based on

Quanti-iT Picogreen quantification (Thermo Fisher Scientific, Wilmington, DE, USA). At least 500 ng of gDNA was bisulfate-converted according to the Zymo EZ DNA methylation kit (Zymo Research, Irvine, CA, USA). Bisulfite-converted DNA was amplified (×1000) to be used on a single BeadChip. The Illumina Infinium HumanMethylation 450K BeadChip was used for blood samples at age 6 (Illumina, San Diego, CA, USA). Since the HumanMethylation 450K BeadChip was no longer available at Illumina, the Illumina Infinium HumanMethylation EPIC BeadChip (850K) was used for blood samples at age 2 (Illumina, San Diego, CA, USA). The amplified DNA was fragmented and hybridized using 50-mer capture probes at CpG loci. For the allele-specific single-base extension assay, primers were extended with a polymerase and labeled nucleotide mix (Two-Color Extension Master Mix) (Illumina, San Diego, CA, USA), stained with the staining and anti-staining reagents, followed by washing and coating. The image was read by the Illumina BeadArray Reader, and image intensities were extracted using Illumina GenomeStudio software. Microarrays were processed at Macrogen (Seoul, Korea).

Raw data were extracted as beta values for each CpG. Beta values were calculated by taking the ratio of the methylated signal intensity over the sum of methylated and unmethylated signals at the 5th carbon (%5mC), which ranged from 0 (no methylation) to 100 (fully methylated). Each signal was subtracted from the background signals from the negative control. Gene-enrichment and functional annotation analyses for each probe list were performed using the Database for Annotation, Visualization, and Integrated Discovery (http://david.abcc.ncifcrf.gov/home.jsp).

2.4.2. Quality control of methylation data

A detection p-value < 0.05 was applied for the filtering criterion. Array CpG probes that had a detection p-value ≥ 0.05 in more than 25% of samples were more likely to be signal to noise and were thus filtered out. Filtered data were normalized using the Beta Mixture Quantile (BMIQ) method (Teschendorff et al. 2013). For the HumanMethylation EPIC BeadChip (850K), CpG sites with "not-available" (NA) values for at least one sample for BMIQ normalization were excluded, leaving 865,688 CpG sites to be analyzed from 866,297 CpG sites in total. For the HumanMethylation 450K BeadChip, CpG sites with NA values of at least one sample for BMIQ normalization were excluded, leaving 460,960 CpG sites for analysis from 485,577 CpG sites in the raw data. Furthermore, CpG sites that corresponded to non-CpG loci or the X or Y chromosome were excluded from further analysis. The exclusion of single nucleotide polymorphism (SNP)-associated CpG sites is described below.

2.4.3. Targeting CpG sites for analysis

Since we were specifically interested in the question of whether DNA methylation mediates the effects of prenatal BPA exposure on child obesity, we targeted CpG sites that were more likely to be involved in obesity rather than scanning the whole epigenome. For the selection of previous EWAS in association with obesity, we searched Pubmed on February 7, 2019, using keywords such as "epigenome-wide association study" and ("BMI" or "obesity") without specific term limits. The selection criteria were EWAS-performed in association with BMI or obesity in healthy adults or children. A total of 24 articles were identified, and we excluded irrelevant articles such as EWAS performed in association with diabetes or in patients with a specific diagnosis such as leukemia or prostate cancer, leaving 12 relevant studies. Using a bibliographic search, we included three additional articles, which resulted in 15 articles for the selection of relevant CpG sites (Al Muftah et al. 2016; Aslibekyan et al. 2015; Campanella et al. 2018; Demerath et al. 2015; Dhana et al. 2018; Huang et al. 2015; Kvaloy et al. 2018; Lin et al. 2017; Rzehak et al. 2017; Sayols-Baixeras et al. 2017; Sharp et al. 2015; Sharp et al. 2017; Wahl et al. 2017; Wilson et al. 2017; Xu et al. 2018) (Table S1). From previous EWAS that investigated the association between DNA methylation and obesity in adults and children, we extracted CpG sites associated with obesity-related parameters such as BMI, BMI Z-score, WC, and fat mass index, resulting in a total of 1100 CpG sites (Table S2).

Duplicate CpG sites reported in more than two studies (210 CpG sites) were excluded, leaving 890 CpG sites (Fig. S1). Among the 890 CpG sites, 135 CpG sites were located within a 0–1 base pair extension from known SNPs, 49 CpG sites had a minor allele frequency (MAF) ≥ 0.05 in SNPs within the target region, and 25 CpG sites corresponded to both occasions, which included up to 209 CpG sites. These CpG sites were excluded from our analysis because the effect of methylation at these CpG sites could be confounded by the effects of the SNPs. Since we matched CpG sites that were present in both the HumanMethylation EPIC BeadChip and the HumanMethylation 450K BeadChip, 87 CpG sites that were only present in either BeadChip were excluded, leaving 594 CpG sites for analysis.

2.5. Covariates

We included covariates in the models to investigate the associations between DNA methylation levels and BMI or BMI Z-score at ages 2, 4, 6, and 8 as follows: mother's age at pregnancy (years), mother's and father's BMI when a child first visited at age 2 (kg/m^2), maternal smoking status (smoked during pregnancy, did not smoke during pregnancy but smoked before pregnancy, and never smoked), mother's educational level (middle school graduate, high school graduate, college graduate, or graduate school attendance), preterm birth (< 37 weeks or ≥ 37 weeks), underweight at birth ($< 2{,}500$ g or $\geq 2{,}500$ g), breastfeeding duration (≥ 6 months or < 6 months), and child's caloric intake (kcal/day) at ages 4, 6, and 8 (unavailable at age 2), which were adjusted at corresponding ages. Prenatal information was obtained using a questionnaire and confirmed by trained research personnel during an interview. Dietary information was obtained from food frequency questionnaires (FFQs) by a dietitian. In a sensitivity analysis, multiple births (singleton or twin/triplet) and maternal alcohol consumption (drank during pregnancy, did not drink during pregnancy but drank before pregnancy, and never drank) were additionally adjusted. The child's urinary BPA level was measured using the same method as for the mother's urinary BPA. Child's urinary BPA levels were available only at age 4, 6, and 8, and was not adjusted in the model because it was considered unrelated with DNA methylation at age 2, thus not a confounder. Nonetheless, we presented the data to compare with maternal BPA levels (Table 1).

For the estimation of cell-type distribution in blood samples, we used an adult leukocyte reference panel, as suggested by Houseman et al. (2012). The Minfi R package was used to estimate the cell-type fraction in each blood sample (Aryee et al. 2014). Fractions of cell types including CD8+ T cells, CD4+ T cells, natural killer (NK) cells, B cells, monocytes, and neutrophils were also adjusted for in the models to examine the association between methylation and child obesity.

2.6. Statistical analysis

Characteristics of the study participants were analyzed using Student's t-test for continuous variables, and a chi-square test or Fisher's exact test for categorical variables to compare two groups.

Fifty-nine samples at ages 2 and 6 were independently run on a single batch. Batch effects by chips and positions were adjusted using ComBat packages in R (Johnson et al. 2007). We used the unadjusted data as we used a single batch, and batch effects by chips or positions were not apparent (Fig. S2a-b). We performed sensitivity analyses of the main findings with batch effects-corrected data.

We searched for differentially methylated CpG sites at ages 2 and 6, significantly different according to prenatal BPA exposure. Prenatal BPA exposure groups were categorized into low (< 2.68 μg/g creatinine) and high (≥ 2.68 μg/g creatinine) exposure group based on the 80th percentile of maternal BPA levels during the 2nd trimester among

Table 1
General characteristics of study participants according to mothers' BPA exposure levels.

Participants	Variables		Low maternal BPA (n = 51)	High maternal BPA (n = 8)	*p*-value
Mother	Age at pregnancy (years)		31.3 ± 3.8	29.5 ± 3.4	0.209
	BMI (kg/m^2)		22.7 ± 3.4	24.0 ± 6.6	0.594
	Educational level	Middle school graduate	0 (0%)	0 (0%)	NA
		High school graduate	7 (13.7%)	2 (25.0%)	
		College graduate	39 (76.5%)	3 (37.5%)	
		Graduate school attendance	5 (9.8%)	3 (37.5%)	
	Urinary BPA in the 2nd trimester of pregnancy (μg/g creatinine)	Urinary BPA	1.34 ± 0.60	7.92 ± 4.97	0.007
		Maximum urinary BPA	2.77	18.38	
	Smoking status during pregnancy	Never smoked	27 (52.9%)	2 (25.0%)	NA
		Smoked tobacco before pregnancy	19 (37.3%)	5 (62.5%)	
		Smoked tobacco during pregnancy	5 (9.8%)	1 (12.5%)	
	Alcohol consumption during pregnancy	Never consumed alcohol	11 (26.2%)	0 (0%)	NA
		Consumed alcohol before pregnancy	20 (47.6%)	3 (50%)	
		Consumed alcohol during pregnancy	11 (26.2%)	3 (50%)	
Father	BMI (kg/m^2)		25.5 ± 3.4	25.0 ± 3.3	0.692
Child	Sex	Male	23 (45.1%)	5 (62.5%)	0.359
		Female	28 (54.9%)	3 (37.5%)	
	Multiple births	Singleton	46 (90.2%)	6 (75%)	0.238
		Multiple*	5 (9.8%)	2 (25%)	
	Preterm (< 37 weeks)	No	46 (90.2%)	5 (62.5%)	0.059
		Yes	5 (9.8%)	3 (37.5%)	
	Underweight at birth (< 2500 g)	No	46 (90.2%)	8 (100%)	0.469
		Yes	5 (9.8%)	0 (0%)	
	Breastfeeding for ≥ 6 months	Yes	26 (51.0%)	4 (50%)	1.000
		No	25 (49.0%)	4 (50%)	
	BPA exposure level	4 years	3.49 ± 2.53	3.68 ± 2.07	0.841
		6 years	3.82 ± 3.26	4.13 ± 2.63	0.799
		8 years	2.05 ± 1.73	0.98 ± 1.36	0.101
	Total calorie intake (kcal/day)	4 years	1,464.3 ± 333.5	1,664.2 ± 481.5	0.144
		6 years	1,574.5 ± 372.9	1,633.1 ± 533.7	0.699
		8 years	1,583.5 ± 397.9	1,568.9 ± 493.7	0.926
	BMI (kg/cm^2)	2 years	16.6 ± 1.2	16.5 ± 1.2	0.782
		4 years	16.0 ± 1.2	15.7 ± 0.7	0.502
		6 years	16.4 ± 1.8	16.3 ± 1.3	0.925
		8 years	17.7 ± 2.7	17.4 ± 1.8	0.745
	BMI Z-score	2 years	−0.06 ± 0.79	−0.18 ± 0.76	0.699
		4 years	0.23 ± 0.88	0.06 ± 0.62	0.588
		6 years	0.31 ± 0.95	0.29 ± 0.84	0.972
		8 years	0.40 ± 1.08	0.31 ± 0.82	0.822
	Cell type fraction	CD4 T cell	0.20 ± 0.04	0.21 ± 0.04	0.613
		CD8 T cell	0.15 ± 0.05	0.18 ± 0.03	0.165
		NK cell	0.04 ± 0.04	0.05 ± 0.05	0.606
		B cell	0.19 ± 0.04	0.15 ± 0.04	0.020
		Monocyte	0.05 ± 0.02	0.04 ± 0.02	0.505
		Neutrophil	0.35 ± 0.09	0.36 ± 0.08	0.796

BPA, bisphenol A; BMI, body mass index.
* Twin (n = 4) and triplet birth (n = 3).

the EDC cohort who visited at 2 years. We also conducted a sensitivity analysis on the basis of various cutoff values of high BPA levels (e.g., 75th, 80th, and 90th percentiles of maternal BPA concentrations).

When examining the distribution of 594 CpG sites by histograms and QQ plots, we found 565 unimodal, 15 bimodal, and 14 trimodal CpG sites. For the unimodal CpG sites, normality was tested using the Shapiro-Wilk test, and differences in the means of methylation levels were compared by prenatal BPA exposure using Student's *t*-test. For the bimodal and trimodal CpG sites, the continuous methylation levels (beta values) were converted to categorical variables [bimodal (0, 1), and trimodal (0, 1) or (0, 2)] by visually inspecting histograms and QQ plots (Fig. S3a-b). A significance level was defined as a false discovery rate (FDR)-corrected *p*-value < 0.10 for multiple comparisons, according to the Benjamini and Hochberg (1995) method.

We constructed a linear mixed model using random intercepts to evaluate the effect of methylation on repeated BMI measurements during the ages of 2–8 years. Methylation levels at cg19196862 were then tested for their association with BMI at 2, 4, 6, and 8 years with linear regression analysis adjusted for the covariates as elaborated earlier. A *p*-value < 0.05 was used for evaluating statistical significance for the regression analyses. Although there was an issue of multiple comparisons of testing the BMI or BMI Z-scores at ages 2, 4, 6, and 8, we considered that it would be difficult to conclude that the associations between obesity-related DNA methylation levels and BMI in children at different ages were independent of each other. Therefore, we did not adjust for multiple comparisons in the regression analysis.

Estimations from the linear regression were multiplied by one standard deviation (SD) at each CpG site to produce the change in the BMI or BMI Z-scores by one SD change of methylation levels. Sex-specific estimates were computed by adding a term for the sex effect as well as an interaction term of the sex effect and DNA methylation (sex × methylation) instead of stratification, while retaining statistical power. We estimated the intraclass correlation coefficient (ICC) to evaluate the stability of methylation between the ages of 2 and 6 years,

and further analyzed the association between longitudinal measures of methylation at ages 2 and 6 years, along with longitudinal BMI or BMI Z-score at ages 2 and 6 years, using a linear mixed model with random intercepts.

We conducted mediation analyses to estimate average causal mediation effects (ACME) and average direct effects (ADE) of methylation on the association between maternal BPA exposure and childhood adiposity. Pathways were specified as in: (1) maternal BPA exposure affecting methylation levels and (2) the methylation levels affecting the BMI at ages 2, 4, 6, and 8, where the mediator was defined as a change in the methylation level at a CpG site. To evaluate whether maternal BPA exposure (log_2BPA) affects methylation levels, linear regression analysis was performed after adjusting for mother's age and BMI, and child's sex. To analyze whether the methylation levels affect BMI or BMI Z-scores at ages 2, 4, 6, and 8 years, linear regression analysis was conducted after adjusting for maternal BPA exposure in addition to the covariates used in the main analysis. The mediation R package was used for the mediation analysis, and nonparametric bootstrapping with 1000 simulations was applied to estimate ACME and ADE. The proportion of mediated indicated the average magnitude of the total mediation effect (ACME + ADE). We used SAS (v9.4) (Cary, NC, USA) and R software (v3.2.1) (R Development Core Team, https://cran.r-project.org/) for statistical analyses.

3. Results

3.1. General characteristics of the participants

Table 1 shows the clinical characteristics of 59 children and the comparison between the two groups according to prenatal BPA exposure (low [n = 51] vs. high [n = 8] BPA exposure). No significant differences were found in parental factors (maternal age at pregnancy and mother's and father's BMI), child factors (sex, multiple births, preterm birth, underweight at birth, breastfeeding duration, total caloric intake, BMI, and BMI Z-score), or the cell type fraction overall (except for the B-cell fraction) between the two groups. The mean maternal urinary BPA levels of the low and high BPA exposure groups were 1.34 ± 0.60 μg/g creatinine (maximum 2.77 μg/g creatinine) and 7.92 ± 4.97 μg/g creatinine (maximum: 18.38 μg/g creatinine), respectively.

Table S3 shows a comparison of clinical characteristics between the 59 children with methylation data analyzed in this substudy and the remaining EDC cohort without methylation data (n = 598). Children included in this substudy showed greater maternal BMI, a higher proportion of maternal smoking or drinking history, lower postnatal BPA exposure, greater child total caloric intake, and greater child BMI or BMI Z-scores at ages 4, 6, and 8 than the remaining 598 children. However, other maternal factors (prenatal BPA exposure, mother's age at pregnancy, and mother's educational level) and child factors (sex, multiple births, preterm birth, underweight at birth, and breastfeeding duration) were not different between the two groups (Table S3).

3.2. Prenatal BPA exposure and DNA methylation

The distribution of 594 targeted CpG sites was inspected; there were 565 unimodal, 15 bimodal (Fig. 1), and 14 trimodal CpG sites. Among 565 CpG sites, 492 CpG sites (87.1%) at age 2, and 498 CpG sites (88.1%) at age 6 showed normal distributions (p-value > 0.05 by Shapiro-Wilk test), respectively (Table S4). When we compared methylation at each CpG site between low and high BPA groups using various cutoff values of high BPA levels (e.g., 75th, 80th, and 90th percentiles of maternal BPA concentrations), the 80th percentile was the most sensitive cutoff value for detecting differences in methylation levels by prenatal BPA exposure (Table S5). Hereafter, we categorized prenatal BPA exposure into two groups (low and high) based on the 80th percentile of maternal BPA concentrations.

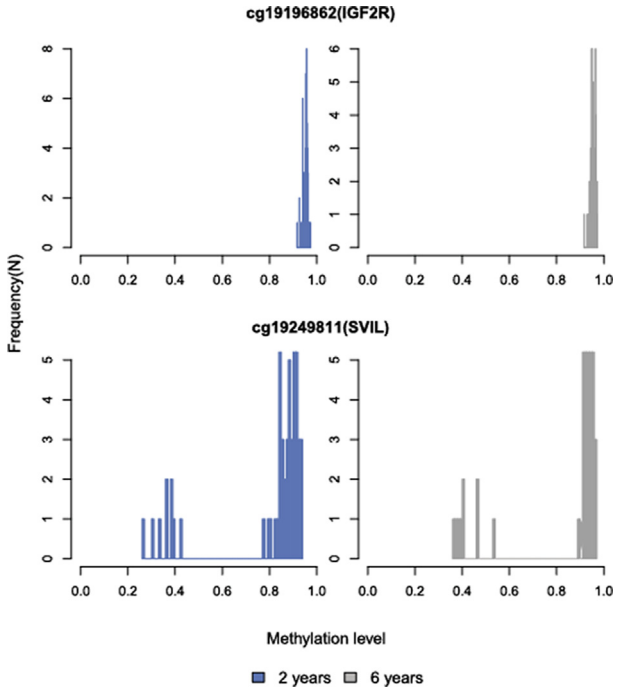

Fig. 1. Distribution of methylation at a unimodal CpG site (cg19196862 (*IGF2R*)) and a bimodal CpG site (cg19249811 (*SVIL*)) at ages 2 and 6 years.

We compared mean levels of methylation at unimodal CpG sites between the prenatal low (n = 51) and high (n = 8) BPA exposure groups. At age 2, methylation at cg19196862 significantly increased in the high BPA exposure group compared to the low BPA exposure group (p-value 0.00030, FDR corrected p-value < 0.10) (Table 2, Table S6, and Fig. 2a), although this difference was not significant at age 6 (Table S7 and Fig. 2a). The change in methylation levels at cg19196862 between ages 2 and 6 (methylation levels at age 6 subtracted by methylation levels at age 2) did not differ between high and low prenatal BPA exposure groups (Table S8). Cg19196862 is located in the 31st exon of *IGF2R* out of 48 exons (Fig. 3).

For bimodal or trimodal CpG sites, Fisher's exact test showed that the distribution of methylation at the bimodal cg19249811 (the first intron of *SVIL* gene) site was different according to prenatal BPA exposure at both ages 2 and 6 years (p-value 0.0012, FDR corrected p-value < 0.10) (Table S9 and Fig. S4).

3.3. DNA methylation and BMI

3.3.1. Methylation at cg19196862 (IGF2R) and BMI during ages 2–8 years

One SD in methylation levels at cg19196862 (*IGF2R*) at age 2 increased BMI significantly by 0.37 (95% CI: 0.06, 0.68), 0.28 (95% CI: 0.00, 0.56), 0.29 (95% CI; −0.11, 0.69), and 0.64 (95% CI; −0.09, 1.37) at ages 2, 4, 6, and 8, respectively, when adjusted for the aforementioned covariates (Table 3 and Fig. 2b). The sex difference was significant in BMI at age 6 (p = 0.048) and 8 years (p-value = 0.046) (Table 3).

The positive association between methylation at cg19196862 (*IGF2R*) at age 2 and BMI was significant only in girls in later ages, by 0.38 (95% CI: 0.00, 0.76) at age 2, 0.37 (95% CI: 0.02, 0.72) at age 4, 0.58 (95% CI: 0.10, 1.05) at age 6, and 1.19 (95% CI: 0.32, 2.07) at age 8, showing increasing effect sizes with age (Table 3 and Fig. 2b). However, the association was not significant in boys, showing decreasing effect sizes with age. Next, we constructed a linear mixed model to evaluate the effect of 2-year-old methylation at cg19196862 (*IGF2R*) on BMI during ages 2–8 years. With 1 SD increase of methylation at cg19196862 (*IGF2R*) at age 2 years, the early childhood BMI

Table 2
Methylation levels of the unimodal and bimodal CpG sites at ages 2 and 6 years by prenatal BPA level.*

		Methylation at 2 years (Mean beta ± SD, or number (%) of children)				Methylation at 6 years (Mean beta ± SD, or number (%) of children)			
Modality	CpG sites	Low prenatal BPA (n = 51)	High prenatal BPA (n = 8)	*p*-value	FDR 0.1	Low prenatal BPA (n = 51)	High prenatal BPA (n = 8)	*p*-value	FDR 0.1
Unimodal	cg19196862 (*IGF2R*)	0.950 ± 0.011	0.959 ± 0.004	3.01×10^{-4}	3.36×10^{-4}	0.954 ± 0.011	0.955 ± 0.011	0.867	0.044
Bimodal*	cg19249811 (*SVIL*) status								
	0	4 (7.84)	5 (62.5)	0.0012	0.0023	4 (7.84)	5 (62.5)	0.012	0.023
	1	47 (92.2)	3 (37.5)			47 (92.2)	3 (37.5)		

BPA, bisphenol A; SD, standard deviation; FDR, false discovery rate.
* 0: relatively low levels of methylation compared to highly methylated status (1).

significantly increased by 0.49 (95% confidence interval [CI]: 0.08, 0.90) in girls, but not in boys. The association between methylation at cg19196862 (*IGF2R*) and BMI Z-score is shown in Table S10 and Fig. S5. ICC for methylation at cg19196862 (*IGF2R*) between ages 2 and 6 was 0.023 (2.3%) (Table S11). Paired methylation and BMI (or BMI Z-scores) at ages 2 and 6 years showed positive associations without significance (Table S11b).

The mediation effect of methylation at cg19196862 (*IGF2R*) at age 2 on the association between prenatal BPA exposure and the BMI or BMI Z-score at ages 2, 4, 6, and 8 were analyzed, respectively, (Fig. 2c and Table S12a-b). In the case of BMI, the indirect effect (average causal mediation effect (ACME)) was marginally significant in early childhood from ages 2 and 4 (p-value < 0.1).

3.3.2. Methylation at cg19249811 (SVIL) and BMI during ages 2–8 years

Methylation at cg19249811 (*SVIL*) at age 2 or 6 was not associated with BMI or BMI Z-score at any ages. Furthermore, repeated measurements of BMI or BMI Z-score were not associated with methylation at cg19249811 (*SVIL*).

3.4. Sensitivity analysis

For sensitivity analysis, we applied various cutoff points such as the 75th, 80th, and 90th percentiles of prenatal BPA exposure levels where methylation at cg19196862 (*IGF2R*) becomes different (Table S5). Using batch effects-corrected data, we performed sensitivity analyses of the association between prenatal BPA and DNA methylation (Table S13) and the association between DNA methylation and BMI or BMI Z-score (Table S14), which showed consistent results. We performed linear regression analysis for the association of methylation level at cg19196862 (*IGF2R*) with the BMI or BMI Z-scores after additionally adjusting for multiple births and alcohol consumption during pregnancy, in addition to the covariates included in the main analysis (Table S15). The effect sizes and p-values were consistent with the main analysis.

4. Discussion

When obesity-associated targeted CpG sites were compared by prenatal BPA exposure, an increase in methylation of cg19196862 (*IGF2R*) levels at age 2 but not 6, and the change in bimodality of the cg19249811 (*SVIL*) site at ages 2 and 6 were found in the high BPA compared to low BPA exposure groups, categorized by the 80th percentile of maternal BPA levels (2.68 μg/g creatinine). Notably, the increased methylation of cg19196862 (*IGF2R*) at age 2 years was persistently associated with BMI during age 2–8 years in girls, but not boys. The mediation effect of 2-year-old methylation at cg19196862 (*IGF2R*) on the association between prenatal BPA exposure and early childhood BMI Z-scores was marginally significant.

Epigenetic dysregulation has been suggested as one of the biological mechanisms of the developmental origin of health and disease (DOHaD). The DOHaD hypothesis suggests that environmental exposures influence developmental programming during critical periods of early life development, leading to permanent changes in disease susceptibility in later life (Waterland and Michels 2007). DNA methylation is a primary epigenetic mechanism involved in DOHaD. During embryonic development, a fertilized cell undergoes epigenetic reprogramming at the early embryo stage, in which DNA methylation is almost completely erased in both the maternal and paternal genomes (Kundakovic and Jaric 2017; Smith et al. 2014). Cell-specific DNA remethylation soon follows (epigenetic programming), whose patterns are inherited from both parents. This dramatic epigenetic reprogramming and programming imply that DNA methylation may be susceptible to changes at earlier stages of development. Although epigenetic marks are stabilized in the later stages of development, they are actively involved in gene expression programming during development. For this

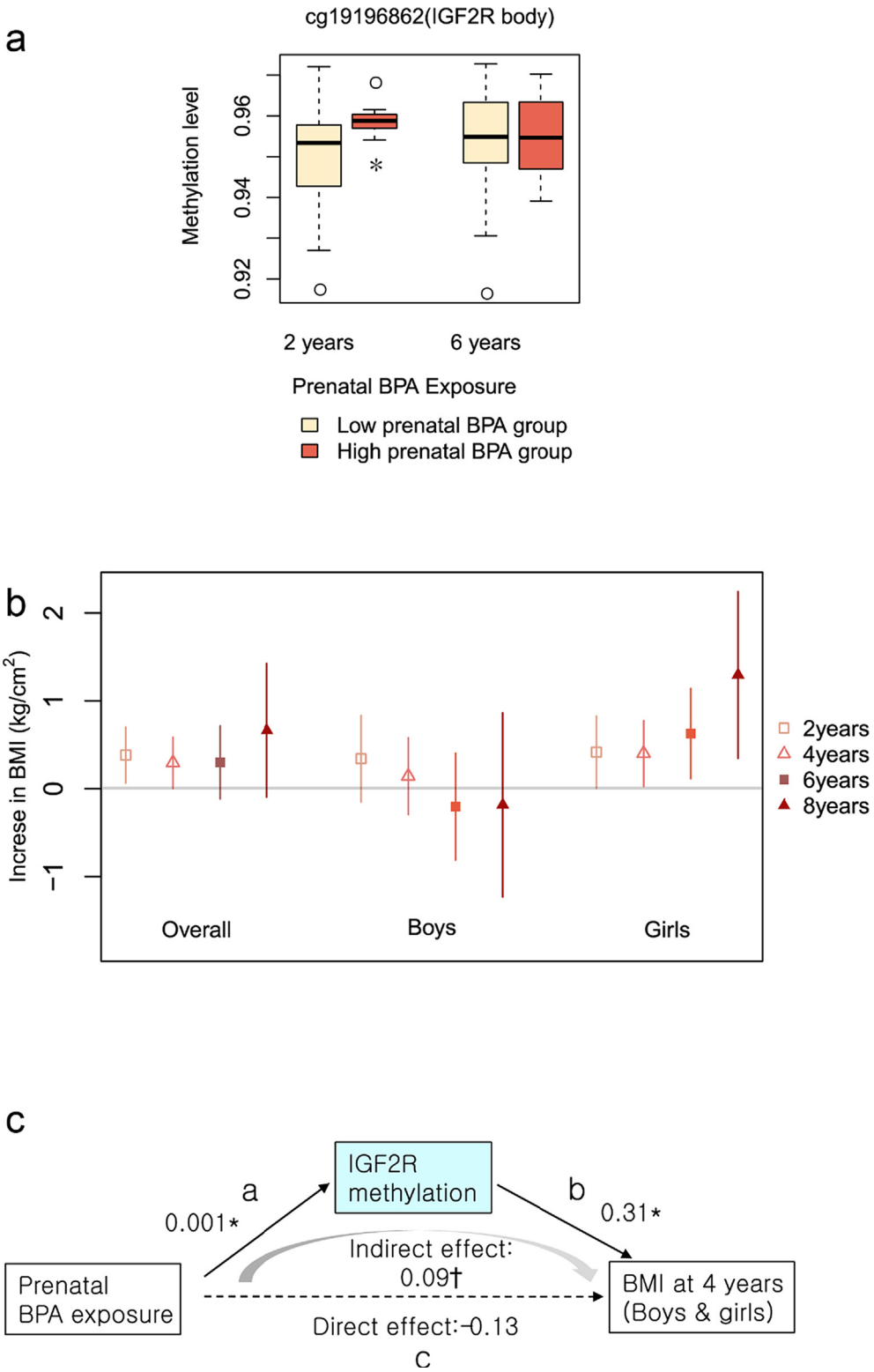

Fig. 2. a. Methylation levels at cg19196862 (*IGF2R* gene) by prenatal BPA (μg/g creatinine) exposure (low: < 80 percentile, high: ≥80 percentile) at ages 2 and 6 years. *FDR corrected *p*-value < 0.10. b. Increase in the BMI (kg/cm^2) and 95% confidence intervals corresponding to one standard deviation increase of methylation levels at cg19196862 (*IGF2R* gene) in boys and girls together, only boys, and only girls at ages 2, 4, 6, and 8 years. The model was adjusted for mother's age at pregnancy (years), mother's and father's BMI when a child first visited at age 2 (kg/m^2), maternal smoking status (smoked during pregnancy, did not smoke during pregnancy but smoked before pregnancy, and never smoked), mother's educational level (middle school graduate, high school graduate, college graduate, or graduate school attendance), preterm birth (< 37 weeks or ≥ 37 weeks), underweight at birth (< 2500 g or ≥ 2500 g), breastfeeding duration (≥6 months or < 6 months), and child's caloric intake (kcal/day) at ages 4, 6, and 8 (unavailable at age 2), and cell type fraction. c. Mediation analysis showing a) the association between prenatal BPA exposure and DNA methylation at cg19196862 at age 2, b) the association between DNA methylation at cg19196862 at age 2 and the BMI at age 4; c) the association between prenatal BPA exposure and the BMI (direct effect: prenatal BPA → child's BMI; indirect effect: prenatal BPA → child's DNA methylation → child's BMI). BMI, body mass index; BPA, bisphenol A; FDR, false discovery rate.

reason, we conducted this study to investigate early-life epigenetic signatures associated with childhood adiposity changes and later obesity development.

ICC for methylation at cg19196862 (*IGF2R*) between ages 2 and 6 was only 0.023 (2.3%). In this study, methylation at cg19196862 (*IGF2R*) site at age 2 years, but not 6 years, was persistently related to BMI during 2–8 years. This implies dynamic changes of DNA methylation during early childhood compared to later stages of development. According to a recent genome-wide methylation study analyzing 783,659 CpG sites, the methylation status in 110,726 CpG sites changed during the first 5 years after birth, while only 460 CpG sites changed during the ages 5–10 (Perez et al. 2019). Importantly, the effect of adversity on DNA methylation changes occurred during very early childhood rather than accumulation or recency of exposures, in that most CpG loci were predicted by early childhood adversity before 3 years of age (Dunn et al. 2019). Therefore, the early stage of child

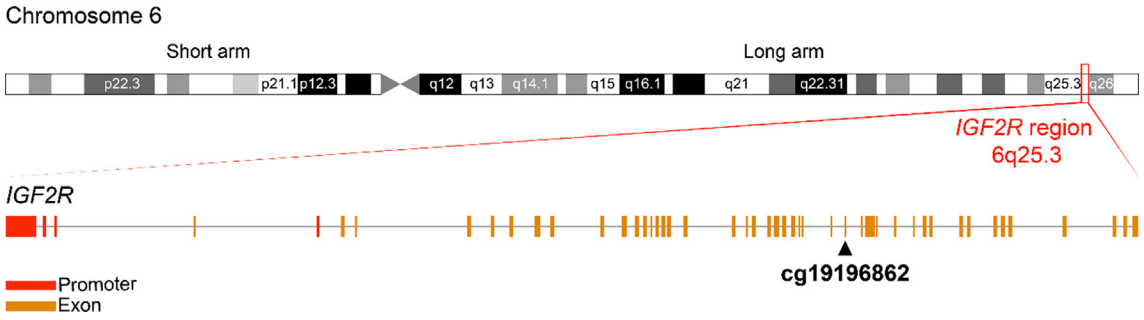

Fig. 3. Chromosome locus and position of cg19196862 in *IGF2R*. Cg19196862 is included in the 31st exon of the *IGF2R* gene out of 48 exons.

development, in which DNA methylation status is still prone to change, would be more susceptible to maternal exposure to environmentally toxic chemicals.

The significant difference in methylation levels at cg19196862 (*IGF2R*) at age 2 by prenatal BPA levels disappeared at age 6. This finding suggests that prenatal toxicant exposure may influence DNA methylation in early childhood, but this effect is mitigated as the methylation profiles become more stabilized with age. As children grow older, more diverse biological and sociological factors during the postnatal period may come into play in determining DNA methylation. Nonetheless, this study points out that prenatal environmental exposure can drive sustained alterations to DNA methylation in infancy and persistent adiposity development, in agreement with the DOHaD hypothesis.

In this study, the greater the methylation of cg19196862 (*IGF2R*), the greater the BMI in early childhood. *IGF2R* regulates the amount of circulating IGF2 by binding, internalizing, and transporting IGF2 into the cell lysosome and degrading it (Hassan 2003). *IGF2* during fetal development has been well documented, and *IGF2* overexpression leads to overgrowth syndromes such as Beckwith-Wiedemann syndrome linked to overgrowth and tumorigenesis (Bergman et al. 2013). IGF2 levels were higher in obese than in normal weight children (Inzaghi et al. 2017) and hypomethylation at *IGF2* increased BMI in children (Acs et al. 2017; Do et al. 2019). The mechanism of *IGF2R* methylation and obesity has not yet been investigated in animal or human studies. We hypothesized that hypermethylation of *IGF2R* may decrease *IGF2R* expression and insufficient degradation of IGF2, leading to an increase in IGF2 levels, Fig. 3 overgrowth and obesity, although the regulatory effect of methylation in the body of the gene is controversial. Further studies are mandatory to prove our hypothesis for the role of cg19196862 (*IGF2R*) methylation on *IGF2R* expression, IGF2 levels, and obesity development.

Methylation status at the bimodal CpG site, cg19249811(*SVIL*), differed by prenatal BPA exposure at ages 2 and 6 years, but it was not associated with BMI in this study. The function of *SVIL* gene is not clearly known, but is reported to be involved in the tight link between actin cytoskeleton and cell membrane (Ghoshdastider et al. 2013). Bimodal and trimodal CpG sites are mostly associated with single nucleotide polymorphisms (SNPs). Genetic loci, usually SNPs, that are associated with different levels of DNA methylation, are called methylation quantitative trait loci (meQTL) (Bell et al. 2011). SNP can cause a bimodal distribution of methylation in CpG sites near SNPs; thus, meQTL needs to be investigated with regard to prenatal environmental exposure and 'genetics × epigenetics' interaction.

In this study, mediation analysis showed indirect effects of BPA on BMI through DNA methylation at *IGF2R* at ages 2 and 4, although only marginally significant (Table S12a-b). The adipogenic effect of BPA has been suggested by several mechanisms: an estrogen receptor-dependent mechanism (Ohlstein et al. 2014), the thyroid receptor/retinoic X receptor or mammalian target of rapamycin signaling pathways (Boucher et al. 2014), increasing 11β-hydroxysteroid dehydrogenase type 1 activity (Wang et al. 2013), and epigenetic mechanisms. There have been suggestions that epigenetic mechanisms are involved in obesogenic effect of BPA. Previous animal studies have shown that developmental BPA exposure changes methylation in candidate genes or global methylation assays (Anderson et al. 2012; Dolinoy et al. 2007). In terms of energy metabolism and body fat, DNA methylation status at Janus kinase-2 (Jak-2), retinoid X receptor (Rxr), regulatory factor x-associated protein (Rfxap), and transmembrane protein 238 (Tmem 238) were identified as mediators using the epigenome-wide discovery platform to evaluate epigenetic alterations in liver tissue from female offspring following prenatal BPA exposure (Anderson et al. 2017).

This study has several limitations. It was limited by the small sample size and lack of genome and gene expression data. Although the different methylation of the cg19196862(*IGF2R*) CpG site by prenatal BPA exposure showed a positive association with early childhood BMI, it needs to be further evaluated which adiposity-associated genetic variants, gene expression, and pathways are affected by *IGF2R* methylation to explain causality and mechanisms. Also, cord blood was not available in this study; thus, we could not compare dynamic methylation status at

Table 3
Association between methylation at cg19196862 (*IGF2R* gene) at age 2 and BMI at ages 2, 4, 6, and 8 (n = 59).*

	Age	Overall		Boys		Girls		Sex difference (Reference: boys)	
	(Years)	Estimate (95% CI)	*p*-value	Estimate (95% CI)	*p*-value	Estimate (95% CI)	*p*-value	Estimate	*p*-value
BMI (kg/m^2)	2	0.37 (0.06, 0.68)	0.024*	0.34 (-0.15, 0.83)	0.184	0.38 (0.00, 0.76)	0.0551	0.04	0.891
	4	0.28 (0.00, 0.56)	0.055	0.14 (-0.30, 0.58)	0.530	0.37 (0.02, 0.72)	0.044*	0.23	0.414
	6	0.29 (-0.11, 0.69)	0.167	−0.21 (-0.82, 0.41)	0.514	0.58 (0.10, 1.05)	0.022*	0.78	0.048*
	8	0.64 (-0.09, 1.37)	0.096	−0.18 (-1.20, 0.86)	0.732	1.19 (0.32, 2.07)	0.011*	1.38	0.046*
2–8†		0.33 (-0.01, 0.67)	0.059	0.05 (-0.48, 0.58)	0.855	0.49 (0.08, 0.90)	0.019*	0.44	0.179

* Adjusted for mother's age at pregnancy (years), mother's and father's BMI when a child first visited at age 2 (kg/m^2), maternal smoking status (smoked during pregnancy, did not smoke during pregnancy but smoked before pregnancy, and never smoked), mother's educational level (middle school graduate, high school graduate, college graduate, or graduate school attendance), preterm birth (< 37 weeks or ≥ 37 weeks), underweight at birth (< 2,500 g or ≥ 2,500 g), breastfeeding duration (≥6 months or < 6 months), and child's caloric intake (kcal/day) at ages 4, 6, and 8 (unavailable at age 2), and cell type fraction.

† Analyzed using a linear mixed model.

birth and early childhood. The direct effect of prenatal BPA on child's BMI was not significant in this sub-study probably due to the small sample size (n = 59); however, prenatal BPA levels showed a positive association with children's BMI in the EDC entire cohort (n = 657) in our previous study (Lim et al. Manuscript submitted). Although the present study did not unveil the epigenetic mechanism directly, we suggested the potential role of DNA methylation in the obesogenic effects of prenatal BPA exposure. Since we targeted CpG sites derived from previous obesity-related EWAS rather than conducting EWAS itself, the possibility of missing novel CpG sites related to BPA exposure cannot be ruled out. EWAS are widely performed in detection of differentially methylated CpG sites or regions associated with phenotype or exposure as a data-driven approach. We attempted a 'hypothesis-driven' analysis based on CpG sites drawn from previous 'data-driven' analyses to investigate epigenetic links between the environmental exposure and clinical outcomes (Lim et al. 2017b).

However, this study was strengthened by its prospective study design including prenatal and postnatal exposure assessment, methylation profiles at ages 2 and 6, and longitudinal outcome measurement from ages 2, 4, 6, and 8 years, which enabled identification of the critical window period of methylation change by prenatal BPA exposure. In addition, sex-specific association between methylation at obesity-related CpG sites and early childhood BMI serves as one answer to explain the suggesting sex-specific effects of prenatal BPA on childhood obesity. To the best of our knowledge, this is the first human study to examine sex-specific effects of methylation affected by prenatal BPA exposure. The study of the mediation effect of methylation at *IGF2R* on the association between prenatal BPA and childhood obesity also contributed to the mechanism underlying the obesogenic effect of BPA. We also had detailed information on obesity-associated covariates, including parental BMI, birth history, breastfeeding, and early childhood caloric intake.

5. Conclusions

In utero exposure to BPA has been indicated as an obesogen in children, and epigenetic regulation has been suggested as an underlying mechanism, with limited evidence in humans. We found a single CpG site that was more highly methylated at ages 2 but not at 6, by prenatal BPA exposure among 594 targeted CpG sites from previous obesity-related EWAS, which was further associated with BMI persistently from ages 2 to 8 years in a sex-dependent manner.

Credit authorship contribution statement

Y.-H.L., Y.-C.H., and Y.-J.C. contributed to conceptualization and primary investigation. Y.-J.C and Y.A.L wrote the original draft, and Y.-H.L. supervised the study. Y.-J.C, J.C. and K.-S.L. were responsible for data curation and formal analysis. C.H.S, B.N.K., and J.I.K contributed to funding acquisition and resources. S.J.P. was responsible for visualization. H.B. and K.B. reviewed and edited the writing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors thank Jin-A Park, Ji-Young Lee, Yumi Choi, and Hyun-ji Lee for their assistance with data collection, and Macrogen for DNA methylation analysis (https://www.macrogen.com/en). The authors also thank Hyungdong Jin at Macrogen for technical help. We would like to thank Editage (www.editage.co.kr) for English language editing. Finally, we greatly appreciate the reviewers for providing valuable advice.

Funding

This study was partially supported by grants from the Environmental Health Center funded by the Ministry of Environment, an R&D Research program funded by the Ministry of Food and Drug Safety (#15162MFDS046), and the Basic Science Research Program through the National Research Foundation funded by the Ministry of Education (2018R1D1A1B07043446 and 2018R1D1A1B07049806), Republic of Korea.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.105929.

References

Acs, O., Peterfia, B., Hollosi, P., Luczay, A., Torok, D., Szabo, A., 2017. Methylation status of CYP27B1 and IGF2 correlate to BMI SDS in children with obesity. Obes. Facts. 10, 353–362. https://doi.org/10.1159/000477462.

Al Muftah, W.A., Al-Shafai, M., Zaghlool, S.B., Visconti, A., Tsai, P.C., Kumar, P., Spector, T., Bell, J., Falchi, M., Suhre, K., 2016. Epigenetic associations of type 2 diabetes and BMI in an Arab population. Clin. Epigenet. 8, 13. https://doi.org/10.1186/s13148-016-0177-6.

Anderson, O.S., Nahar, M.S., Faulk, C., Jones, T.R., Liao, C., Kannan, K., Weinhouse, C., Rozek, L.S., Dolinoy, D.C., 2012. Epigenetic responses following maternal dietary exposure to physiologically relevant levels of bisphenol A. Environ. Mol. Mutagen. 53, 334–342. https://doi.org/10.1002/em.21692.

Anderson, O.S., Kim, J.H., Peterson, K.E., Sanchez, B.N., Sant, K.E., Sartor, M.A., Weinhouse, C., Dolinoy, D.C., 2017. Novel epigenetic biomarkers mediating bisphenol A exposure and metabolic phenotypes in female mice. Endocrinology 158, 31–40. https://doi.org/10.1210/en.2016-1441.

Aryee, M.J., Jaffe, A.E., Corrada-Bravo, H., Ladd-Acosta, C., Feinberg, A.P., Hansen, K.D., Irizarry, R.A., 2014. Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. Bioinformatics 30, 1363–1369. https://doi.org/10.1093/bioinformatics/btu049.

Aslibekyan, S., Demerath, E.W., Mendelson, M., Zhi, D., Guan, W., Liang, L., Sha, J., Pankow, J.S., Liu, C., Irvin, M.R., Fornage, M., Hidalgo, B., Lin, L.A., Thibeault, K.S., Bressler, J., Tsai, M.Y., Grove, M.L., Hopkins, P.N., Boerwinkle, E., Borecki, I.B., Ordovas, J.M., Levy, D., Tiwari, H.K., Absher, D.M., Arnett, D.K., 2015. Epigenome-wide study identifies novel methylation loci associated with body mass index and waist circumference. Obesity (Silver Spring). 23, 1493–1501. https://doi.org/10.1002/oby.21111.

Balakrishnan, B., Henare, K., Thorstensen, E.B., Ponnampalam, A.P., Mitchell, M.D., 2010. Transfer of bisphenol A across the human placenta. Am. J. Obstet. Gynecol. 202: 393.e391–397. http://doi.org/10.1016/j.ajog.2010.01.025.

Bell, J.T., Pai, A.A., Pickrell, J.K., Gaffney, D.J., Pique-Regi, R., Degner, J.F., Gilad, Y., Pritchard, J.K., 2011. DNA methylation patterns associate with genetic and gene expression variation in HapMap cell lines. Genome Biol. 12, R10. https://doi.org/10.1186/gb-2011-12-1-r10.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. Roy. Statist. Soc. B. 57, 289–300.

Bergman, D., Halje, M., Nordin, M., Engstrom, W., 2013. Insulin-like growth factor 2 in development and disease: a mini-review. Gerontology 59, 240–249. https://doi.org/10.1159/000343995.

Boucher, J.G., Husain, M., Rowan-Carroll, A., Williams, A., Yauk, C.L., Atlas, E., 2014. Identification of mechanisms of action of bisphenol a-induced human preadipocyte differentiation by transcriptional profiling. Obesity (Silver Spring). 22, 2333–2343. https://doi.org/10.1002/oby.20848.

Braun, J.M., 2017. Early-life exposure to EDCs: role in childhood obesity and neurodevelopment. Nat. Rev. Endocrinol. 13, 161–173. https://doi.org/10.1038/nrendo.2016.186.

Calafat, A.M., Kuklenyik, Z., Reidy, J.A., Caudill, S.P., Ekong, J., Needham, L.L., 2005. Urinary concentrations of bisphenol A and 4-nonylphenol in a human reference population. Environ. Health Perspect. 113, 391–395. https://doi.org/10.1289/ehp.7534.

Campanella, G., Gunter, M.J., Polidoro, S., Krogh, V., Palli, D., Panico, S., Sacerdote, C., Tumino, R., Fiorito, G., Guarrera, S., Iacoviello, L., Bergdahl, I.A., Melin, B., Lenner, P., de Kok, T., Georgiadis, P., Kleinjans, J.C.S., Kyrtopoulos, S.A., Bueno-de-Mesquita, H.B., Lillycrop, K.A., May, A.M., Onland-Moret, N.C., Murray, R., Riboli, E., Verschuren, M., Lund, E., Mode, N., Sandanger, T.M., Fiano, V., Trevisan, M., Matullo, G., Froguel, P., Elliott, P., Vineis, P., Chadeau-Hyam, M., 2018. Epigenome-wide association study of adiposity and future risk of obesity-related diseases. Int. J. Obes. (Lond.) 42, 2022–2035. https://doi.org/10.1038/s41366-018-0064-7.

Demerath, E.W., Guan, W., Grove, M.L., Aslibekyan, S., Mendelson, M., Zhou, Y.H., Hedman, A.K., Sandling, J.K., Li, L.A., Irvin, M.R., Zhi, D., Deloukas, P., Liang, L., Liu, C., Bressler, J., Spector, T.D., North, K., Li, Y., Absher, D.M., Levy, D., Arnett, D.K.,

Fornage, M., Pankow, J.S., Boerwinkle, E., 2015. Epigenome-wide association study (EWAS) of BMI, BMI change and waist circumference in African American adults identifies multiple replicated loci. Hum. Mol. Genet. 24, 4464–4479. https://doi.org/10.1093/hmg/ddv161.

Dhana, K., Braun, K.V.E., Nano, J., Voortman, T., Demerath, E.W., Guan, W., Fornage, M., van Meurs, J.B.J., Uitterlinden, A.G., Hofman, A., Franco, O.H., Dehghan, A., 2018. An epigenome-wide association study of obesity-related traits. Am. J. Epidemiol. 187, 1662–1669. https://doi.org/10.1093/aje/kwy025.

Do, E.K., Zucker, N.L., Huang, Z.Y., Schechter, J.C., Kollins, S.H., Maguire, R.L., Murphy, S.K., Hoyo, C., Fuemmeler, B.F., 2019. Associations between imprinted gene differentially methylated regions, appetitive traits and body mass index in children. Pediatr. Obes. 14, e12454. https://doi.org/10.1111/ijpo.12454.

Dolinoy, D.C., Huang, D., Jirtle, R.L., 2007. Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. Proc. Natl. Acad. Sci. U. S. A. 104, 13056–13061. https://doi.org/10.1073/pnas.0703739104.

Dunn, E.C., Soare, T.W., Zhu, Y., Simpkin, A.J., Suderman, M.J., Klengel, T., Smith, A., Ressler, K.J., Relton, C.L., 2019. Sensitive periods for the effect of childhood adversity on DNA methylation: results from a prospective, longitudinal study. Biol. Psychiatry. 85, 838–849. https://doi.org/10.1016/j.biopsych.2018.12.023.

Ghoshdastider, U., Popp, D., Fau-Burtnick, L.D., Burtnick Ld Fau - Robinson R.C., Robinson, R.C., 2013. The expanding superfamily of gelsolin homology domain proteins. Cytoskeleton (Hoboken). 70, 775–795.

Goodrich, J.M., Dolinoy, D.C., Sanchez, B.N., Zhang, Z., Meeker, J.D., Mercado-Garcia, A., Solano-Gonzalez, M., Hu, H., Tellez-Rojo, M.M., Peterson, K.E., 2016. Adolescent epigenetic profiles and environmental exposures from early life through peri-adolescence. Environ Epigenet. 2, 1–12. https://doi.org/10.1093/eep/dvw018.

Harley, K.G., Aguilar, Schall R., Chevrier, J., Tyler, K., Aguirre, H., Bradman, A., Holland, N.T., Lustig, R.H., Calafat, A.M., Eskenazi, B., 2013. Prenatal and postnatal bisphenol A exposure and body mass index in childhood in the CHAMACOS cohort. Environ. Health Perspect. 121, 514–520. https://doi.org/10.1289/ehp.1205548.

Hassan, A.B., 2003. Keys to the hidden treasures of the mannose 6-phosphate/insulin-like growth factor 2 receptor. Am. J. Pathol. 162, 3–6. https://doi.org/10.1016/s0002-9440(10)63791-1.

Hoepner, L.A., Whyatt, R.M., Widen, E.M., Hassoun, A., Oberfield, S.E., Mueller, N.T., Diaz, D., Calafat, A.M., Perera, F.P., Rundle, A.G., 2016. Bisphenol A and Adiposity in an Inner-City Birth Cohort. Environ. Health Perspect. 124, 1644–1650. https://doi.org/10.1289/ehp205.

Houseman, E.A., Accomando, W.P., Koestler, D.C., Christensen, B.C., Marsit, C.J., Nelson, H.H., Wiencke, J.K., Kelsey, K.T., 2012. DNA methylation arrays as surrogate measures of cell mixture distribution. BMC Bioinform. 13, 86. https://doi.org/10.1186/1471-2105-13-86.

Huang, R.C., Garratt, E.S., Pan, H., Wu, Y., Davis, E.A., Barton, S.J., Burdge, G.C., Godfrey, K.M., Holbrook, J.D., Lillycrop, K.A., 2015. Genome-wide methylation analysis identifies differentially methylated CpG loci associated with severe obesity in childhood. Epigenetics 10, 995–1005. https://doi.org/10.1080/15592294.2015.1080411.

Inzaghi, E., Baldini, Ferroli B., Fintini, D., Grossi, A., Nobili, V., Cianfarani, S., 2017. Insulin-like growth factors and metabolic syndrome in obese children. Horm. Res. Paediatr. 87, 400–404. https://doi.org/10.1159/000477241.

Johnson, W.E., Li, C., Rabinovic, A., 2007. Adjusting batch effects in microarray expression data using empirical Bayes methods. Biostatistics 8, 118–127. https://doi.org/10.1093/biostatistics/kxj037.

Junge, K.M., Leppert, B., Jahreis, S., Wissenbach, D.K., Feltens, R., Grutzmann, K., Thurmann, L., Bauer, T., Ishaque, N., Schick, M., Bewerunge-Hudler, M., Roder, S., Bauer, M., Schulz, A., Borte, M., Landgraf, K., Korner, A., Kiess, W., von Bergen, M., Stangl, G.I., Trump, S., Eils, R., Polte, T., Lehmann, I., 2018. MEST mediates the impact of prenatal bisphenol A exposure on long-term body weight development. Clin. Epigenetics. 10, 58. https://doi.org/10.1186/s13148-018-0478-z.

Kim, K.N., Lim, Y.H., Shin, C.H., Lee, Y.A., Kim, B.N., Kim, J.I., Hwang, I.G., Hwang, M.S., Suh, J.H., Hong, Y.C., 2018. Cohort Profile: The Environment and Development of Children (EDC) study: a prospective children's cohort. Int. J. Epidemiol. 47, 1049–1050f. https://doi.org/10.1093/ije/dyy070.

Kundakovic, M., Jaric, I., 2017. The Epigenetic Link between Prenatal Adverse Environments and Neurodevelopmental Disorders. Genes (Basel). 8. https://doi.org/10.3390/genes8030104.

Kvaloy, K., Page, C.M., Holmen, T.L., 2018. Epigenome-wide methylation differences in a group of lean and obese women - A HUNT Study. Sci. Rep. 8, 16330. https://doi.org/10.1038/s41598-018-34003-8.

Lim, Y.H., Bae, S., Kim, B.N., Shin, C.H., Lee, Y.A., Kim, J.I., Hong, Y.C., 2017a. Prenatal and postnatal bisphenol A exposure and social impairment in 4-year-old children. Environ. Health. 16, 79. https://doi.org/10.1186/s12940-017-0289-2.

Lim, Y.H., Han, C., Bae, S., Hong, Y.C., 2017b. Modulation of blood pressure in response to low ambient temperature: The role of DNA methylation of zinc finger genes. Environ. Res. 153, 106–111. https://doi.org/10.1016/j.envres.2016.11.019.

Lim Y.H., Lee Y.A., Choi Y.-J., Shin C.H., Lee D., Kim B.-N., Kim J.I., Hong Y.C., Manuscript submitted. Sex-specific effects of bisphenol A exposure on body mass index in early childhood: a prospective cohort study (submitted for publication).

Lin, X., Lim, I.Y., Wu, Y., Teh, A.L., Chen, L., Aris, I.M., Soh, S.E., Tint, M.T., MacIsaac, J.L., Morin, A.M., Yap, F., Tan, K.H., Saw, S.M., Kobor, M.S., Meaney, M.J., Godfrey, K.M., Chong, Y.S., Holbrook, J.D., Lee, Y.S., Gluckman, P.D., Karnani, N., 2017. Developmental pathways to adiposity begin before birth and are influenced by genotype, prenatal environment and epigenome. BMC Med. 15, 50. https://doi.org/10.1186/s12916-017-0800-1.

Moon, J.S., Lee, S.Y., Nam, C.M., Choi, J.M., Choe, B.K., Weo, J.W., Oh, K., Jang, M.-J., Hwang, S.S., Yoo, M.H., 2008. 2007 Korean National Growth Charts: review of developmental process and an outlook. Kor J Pediatrics. 51, 1–25.

Myridakis, A., Fthenou, E., Balaska, E., Vakinti, M., Kogevinas, M., Stephanou, E.G., 2015. Phthalate esters, parabens and bisphenol-A exposure among mothers and their children in Greece (Rhea cohort). Environ. Int. 83, 1–10. https://doi.org/10.1016/j.envint.2015.05.014.

Ohlstein, J.F., Strong, A.L., McLachlan, J.A., Gimble, J.M., Burow, M.E., Bunnell, B.A., 2014. Bisphenol A enhances adipogenic differentiation of human adipose stromal/stem cells. J. Mol. Endocrinol. 53, 345–353. https://doi.org/10.1530/jme-14-0052.

Perez, R.F., Santamarina, P., Tejedor, J.R., Urdinguio, R.G., Alvarez-Pitti, J., Redon, P., Fernandez, A.F., Fraga, M.F., Lurbe, E., 2019. Longitudinal genome-wide DNA methylation analysis uncovers persistent early-life DNA methylation changes. J. Transl. Med. 17, 15. https://doi.org/10.1186/s12967-018-1751-9.

Romano, M.E., Webster, G.M., Vuong, A.M., Thomas, Zoeller R., Chen, A., Hoofnagle, A.N., Calafat, A.M., Karagas, M.R., Yolton, K., Lanphear, B.P., Braun, J.M., 2015. Gestational urinary bisphenol A and maternal and newborn thyroid hormone concentrations: the HOME Study. Environ. Res. 138, 453–460. https://doi.org/10.1016/j.envres.2015.03.003.

Rzehak, P., Covic, M., Saffery, R., Reischl, E., Wahl, S., Grote, V., Weber, M., Xhonneux, A., Langhendries, J.P., Ferre, N., Closa-Monasterolo, R., Escribano, J., Verduci, E., Riva, E., Socha, P., Gruszfeld, D., Koletzko, B., 2017. DNA-methylation and body composition in preschool children: epigenome-wide-analysis in the european childhood obesity project (CHOP)-study. Sci. Rep. 7, 14349. https://doi.org/10.1038/s41598-017-13099-4.

Sayols-Baixeras, S., Subirana, I., Fernandez-Sanles, A., Senti, M., Lluis-Ganella, C., Marrugat, J., Elosua, R., 2017. DNA methylation and obesity traits: An epigenome-wide association study. The REGICOR study. Epigenetics 12, 909–916. https://doi.org/10.1080/15592294.2017.1363951.

Sharp, G.C., Lawlor, D.A., Richmond, R.C., Fraser, A., Simpkin, A., Suderman, M., Shihab, H.A., Lyttleton, O., McArdle, W., Ring, S.M., Gaunt, T.R., Davey, Smith G., Relton, C.L., 2015. Maternal pre-pregnancy BMI and gestational weight gain, offspring DNA methylation and later offspring adiposity: findings from the Avon Longitudinal Study of Parents and Children. Int. J. Epidemiol. 44, 1288–1304. https://doi.org/10.1093/ije/dyv042.

Sharp, G.C., Salas, L.A., Monnereau, C., Allard, C., Yousefi, P., Everson, T.M., Bohlin, J., Xu, Z., Huang, R.C., Reese, S.E., Xu, C.J., Baiz, N., Hoyo, C., Agha, G., Roy, R., Holloway, J.W., Ghantous, A., Merid, S.K., Bakulski, K.M., Kupers, L.K., Zhang, H., Richmond, R.C., Page, C.M., Duijts, L., Lie, R.T., Melton, P.E., Vonk, J.M., Nohr, E.A., Williams-DeVane, C., Huen, K., Rifas-Shiman, S.L., Ruiz-Arenas, C., Gonseth, S., Rezwan, F.I., Herceg, Z., Ekstrom, S., Croen, L., Falahi, F., Perron, P., Karagas, M.R., Quraishi, B.M., Suderman, M., Magnus, M.C., Jaddoe, V.W.V., Taylor, J.A., Anderson, D., Zhao, S., Smit, H.A., Josey, M.J., Bradman, A., Baccarelli, A.A., Bustamante, M., Haberg, S.E., Pershagen, G., Hertz-Picciotto, I., Newschaffer, C., Corpeleijn, E., Bouchard, L., Lawlor, D.A., Maguire, R.L., Barcellos, L.F., Davey, Smith G., Eskenazi, B., Karmaus, W., Marsit, C.J., Hivert, M.F., Snieder, H., Fallin, M.D., Melen, E., Munthe-Kaas, M.C., Arshad, H., Wiemels, J.L., Annesi-Maesano, I., Vrijheid, M., Oken, E., Holland, N., Murphy, S.K., Sorensen, T.I.A., Koppelman, G.H., Newnham, J.P., Wilcox, A.J., Nystad, W., London, S.J., Felix, J.F., Relton, C.L., 2017. Maternal BMI at the start of pregnancy and offspring epigenome-wide DNA methylation: findings from the pregnancy and childhood epigenetics (PACE) consortium. Hum. Mol. Genet. 26, 4067–4085. https://doi.org/10.1093/hmg/ddx290.

Smith, Z.D., Chan, M.M., Humm, K.C., Karnik, R., Mekhoubad, S., Regev, A., Eggan, K., Meissner, A., 2014. DNA methylation dynamics of the human preimplantation embryo. Nature. 511, 611–615. https://doi.org/10.1038/nature13581.

Teschendorff, A.E., Marabita, F., Lechner, M., Bartlett, T., Tegner, J., Gomez-Cabrero, D., Beck, S., 2013. A beta-mixture quantile normalization method for correcting probe design bias in Illumina Infinium 450 k DNA methylation data. Bioinformatics. 29, 189–196. https://doi.org/10.1093/bioinformatics/bts680.

Vafeiadi, M., Roumeliotaki, T., Myridakis, A., Chalkiadaki, G., Fthenou, E., Dermitzaki, E., Karachaliou, M., Sarri, K., Vassilaki, M., Stephanou, E.G., Kogevinas, M., Chatzi, L., 2016. Association of early life exposure to bisphenol A with obesity and cardiometabolic traits in childhood. Environ. Res. 146, 379–387. https://doi.org/10.1016/j.envres.2016.01.017.

van Esterik, J.C., Dolle, M.E., Lamoree, M.H., van Leeuwen, S.P., Hamers, T., Legler, J., van der Ven, L.T., 2014. Programming of metabolic effects in C57BL/6JxFVB mice by exposure to bisphenol A during gestation and lactation. Toxicology. 321, 40–52. https://doi.org/10.1016/j.tox.2014.04.001.

van Esterik, J.C., Vitins, A.P., Hodemaekers, H.M., Kamstra, J.H., Legler, J., Pennings, J.L., Steegenga, W.T., Lute, C., Jelinek, J., Issa, J.P., Dolle, M.E., van der Ven, L.T., 2015. Liver DNA methylation analysis in adult female C57BL/6JxFVB mice following perinatal exposure to bisphenol A. Toxicol. Lett. 232, 293–300. https://doi.org/10.1016/j.toxlet.2014.10.021.

Vandenberg, L.N., Hauser, R., Marcus, M., Olea, N., Welshons, W.V., 2007. Human exposure to bisphenol A (BPA). Reprod. Toxicol. 24, 139–177. https://doi.org/10.1016/j.reprotox.2007.07.010.

Vandenberg, L.N., Chahoud, I., Heindel, J.J., Padmanabhan, V., Paumgartten, F.J., Schoenfelder, G., 2010. Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. Environ. Health Perspect. 118, 1055–1070. https://doi.org/10.1289/ehp.0901716.

von Goetz, N., Wormuth, M., Scheringer, M., Hungerbuhler, K., 2010. Bisphenol a: how the most relevant exposure sources contribute to total consumer exposure. Risk Anal. 30, 473–487. https://doi.org/10.1111/j.1539-6924.2009.01345.x.

Wahl, S., Drong, A., Lehne, B., Loh, M., Scott, W.R., Kunze, S., Tsai, P.C., Ried, J.S., Zhang, W., Yang, Y., Tan, S., Fiorito, G., Franke, L., Guarrera, S., Kasela, S., Kriebel, J., Richmond, R.C., Adamo, M., Afzal, U., Ala-Korpela, M., Albetti, B., Ammerpohl, O., Apperley, J.F., Beekman, M., Bertazzi, P.A., Black, S.L., Blancher, C., Bonder, M.J., Brosch, M., Carstensen-Kirberg, M., de Craen, A.J., de Lusignan, S., Dehghan, A., Elkalaawy, M., Fischer, K., Franco, O.H., Gaunt, T.R., Hampe, J., Hashemi, M.,

Isaacs, A., Jenkinson, A., Jha, S., Kato, N., Krogh, V., Laffan, M., Meisinger, C., Meitinger, T., Mok, Z.Y., Motta, V., Ng, H.K., Nikolakopoulou, Z., Nteliopoulos, G., Panico, S., Pervjakova, N., Prokisch, H., Rathmann, W., Roden, M., Rota, F., Rozario, M.A., Sandling, J.K., Schafmayer, C., Schramm, K., Siebert, R., Slagboom, P.E., Soininen, P., Stolk, L., Strauch, K., Tai, E.S., Tarantini, L., Thorand, B., Tigchelaar, E.F., Tumino, R., Uitterlinden, A.G., van Duijn, C., van Meurs, J.B., Vineis, P., Wickremasinghe, A.R., Wijmenga, C., Yang, T.P., Yuan, W., Zhernakova, A., Batterham, R.L., Smith, G.D., Deloukas, P., Heijmans, B.T., Herder, C., Hofman, A., Lindgren, C.M., Milani, L., van der Harst, P., Peters, A., Illig, T., Relton, C.L., Waldenberger, M., Jarvelin, M.R., Bollati, V., Soong, R., Spector, T.D., Scott, J., McCarthy, M.I., Elliott, P., Bell, J.T., Matullo, G., Gieger, C., Kooner, J.S., Grallert, H., Chambers, J.C., 2017. Epigenome-wide association study of body mass index, and the adverse outcomes of adiposity. Nature. 541, 81–86. https://doi.org/10.1038/nature20784.

Wang, J., Sun, B., Hou, M., Pan, X., Li, X., 2013. The environmental obesogen bisphenol A promotes adipogenesis by increasing the amount of 11beta-hydroxysteroid dehydrogenase type 1 in the adipose tissue of children. Int. J. Obes. (Lond.) 37, 999–1005. https://doi.org/10.1038/ijo.2012.173.

Waterland, R.A., Michels, K.B., 2007. Epigenetic epidemiology of the developmental origins hypothesis. Annu. Rev. Nutr. 27, 363–388. https://doi.org/10.1146/annurev.nutr.27.061406.093705.

Wilson, L.E., Harlid, S., Xu, Z., Sandler, D.P., Taylor, J.A., 2017. An epigenome-wide study of body mass index and DNA methylation in blood using participants from the Sister Study cohort. Int. J. Obes. (Lond.) 41, 194–199. https://doi.org/10.1038/ijo.2016.184.

Xu, K., Zhang, X., Wang, Z., Hu, Y., Sinha, R., 2018. Epigenome-wide association analysis revealed that SOCS3 methylation influences the effect of cumulative stress on obesity. Biol. Psychol. 131, 63–71. https://doi.org/10.1016/j.biopsycho.2016.11.001.

Yang, M., Kim, S.Y., Lee, S.M., Chang, S.S., Kawamoto, T., Jang, J.Y., Ahn, Y.O., 2003. Biological monitoring of bisphenol a in a Korean population. Arch. Environ. Contam. Toxicol. 44, 546–551. https://doi.org/10.1007/s00244-002-2124-0.